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Metal Ion Catalysed Adenosine-5′-triphosphate Hydrolysis: Part I—Cu(II) Catalysed Hydrolysis

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The kinetics of the cupric ion catalysed hydrolysis of adenosine-5′-triphosphate (ATP) in aqueous solutions has been studied at 46·0°, 56·0° and 64·0°, over the broad *p*H-range 3·0-8·0, and $\mu = 0{\cdot}1$ (KNO_3). *p*H-rate profiles have been analysed and the overall rate constants have been resolved into specific rate constants relating to the various Cu : ATP chelate species in solution. Activation parameters $\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$ and $\Delta F^{\ddagger}$, for the specific rate constants of the various chelate species are reported. The possible mechanisms for the hydrolysis of the various ATP ionic species in the absence and the presence of Cu(II) ions are discussed.

IN a previous publication[1] the kinetics of the nonenzymic hydrolysis of adenosine-5′-triphosphate (ATP) in the absence of metal ions has been reported. However, the presence of bivalent metal ions are found to be essential in the *in vivo* enzymic hydrolysis reactions of ATP[2,3]. An understanding of the mechanism of metal ion catalysis in simple nonenzymic systems will be of much help in interpreting the function and role of metal ions in enzymic ATP reactions. It was therefore considered important to carry out a detailed physico-chemical study of the effect of a few bivalent metal ions on the nonenzymic hydrolysis of ATP in aqueous solutions, as a function of *p*H and temperature. Earlier investigators in this field[4-11] have investigated the effect of Mg(II), Ca(II) and Ba(II) ions on the rate of ATP and ADP hydrolysis. The study was extended to metal ions less basic than the alkaline earths by Liebecq[12], Lowenstien[13] and Westheimer[14]. However, these investigations were only qualitative or semiquantitative in nature and various buffers known to complex with metal ions[15] were used to maintain a constant *p*H. Schneider and Brintzinger[16] have also reported the effect of Cu(II) ions on the ATP hydrolysis. In this paper are reported the results of a detailed, physico-chemical study of the effect of Cu(II) ions on the nonenzymic hydrolysis of ATP at 46°, 56° and 64° over the broad *p*H range of 3-8 and $\mu = 0{\cdot}1$ (KNO_3).

Materials and Methods

A chromatographically pure crystalline adenosine-5′-triphosphate dipotassium salt (from Mann Research Laboratories, USA) was used. The aqueous solution $Cu(NO_3)_2$ (AR) was standardized using EDTA (disodium salt) by the procedure outlined by Schwarzenbach[17].

The experimental procedure used was essentially the same as that used for the uncatalysed study[1] To maintain a constant temperature of 56°, 64° and 78°, acetone, methanol and ethanol respectively were used along with the experimental set-up described[1]. For work at 46°, a double-walled glass cell serviced by a constant temperature water-bath at $46° \pm 0{\cdot}1°$ was used. The *p*H of the solution was initially adjusted to the required *p*H, by the addition of enough acid or alkali. Any change in *p*H during the course of the kinetic runs was also adjusted by adding acid or alkali. Buffers were not used to maintain a constant *p*H, since the basic component of most buffers are known to complex with Cu(II) ions[15].

Kinetic runs were carried out with equimolar amounts of Cu(II) and ATP ($\sim 2{\cdot}0 \times 10^{-3} M$), over the *p*H range 3-8 at 46°, 56° and 64°. First order rate constants were evaluated in the initial stages of the hydrolysis reaction from plots of $\log a\,(a-x)$ against t (where a = initial concentration of ATP and x = amount reacted at time t). By restricting the kinetic study to the initial stages of the reaction, complications resulting from further hydrolysis of products was eliminated. All calculations were carried out with an IBM 370/155 computer.

Results

In the previous publication[1], kinetic data for the uncatalysed ATP hydrolysis was reported at 56° and 64°. This work has now been extended by studying the kinetics of the uncatalysed ATP hydrolysis at 78° also. Separate values for the specific rate constants of the monoprotonated species, $HATP^{3-}$ were reported[1], one for the *p*H region 3-7 and the other for the *p*H region 7-9. As the *p*H region 6·0-7·5 is very sensitive to slight changes in *p*H of the solution, the kinetic runs in this *p*H region were repeated, and the first order rate constants obtained for the uncatalysed ATP hydrolyses are given in Table 1. Using this data recalculation of the specific rate constants in the

TABLE 1 — EXPERIMENTAL AND CALCULATED RATE CONSTANTS FOR THE UNCATALYSED ATP HYDROLYSIS

[μ = 0·1 (KNO_3)]

pH	Rate constant × 10^4 (min^{-1}) at					
	56°		64°		78°	
	Expl	Calc.	Expl	Calc.	Expl	Calc.
3·0	1·89	1·84	5·69	5·54	33·37	34·86
3·5	1·80	1·78	5·26	5·22	30·07	31·51
4·0	1·70	1·72	4·87	4·91	26·24	27·66
4·5	1·67	1·66	4·76	4·63	23·66	25·01
5·5	1·52	1·51	4·14	3·98	20·58	21·83
6·0	1·44	1·33	3·80	3·59	19·30	18·32
6·5	1·12	0·99	2·85	2·60	13·33	12·78
7·0	0·76	0·66	1·87	1·64	7·95	7·43
7·5	0·55	0·47	1·25	1·09	4·77	4·42
8·0	0·37	0·39	0·92	0·87	3·53	3·24
9·0	0·36	0·36	0·74	0·77	2·87	2·70

TABLE 2 — SPECIFIC RATE CONSTANTS (min^{-1}) FOR THE UNCATALYSED AND Cu(II) CATALYSED ATP HYDROLYSIS

[μ = 0·1 (KNO_3)]

ATP species	46°	56°	64°	78°
H_2ATP^{2-}		$1·87 \times 10^{-4}$ (±0·10)	$5·75 \times 10^{-4}$ (±0·24)	$3·76 \times 10^{-3}$ (±0·15)
$HATP^{3-}$		$1·63 \times 10^{-4}$ (±0·06)	$4·50 \times 10^{-4}$ (±0·15)	$2·37 \times 10^{-3}$ (±0·14)
ATP^{4-}		$3·56 \times 10^{-5}$ (±0·23)	$7·55 \times 10^{-5}$ (±0·34)	$2·63 \times 10^{-4}$ (±0·25)
Cu: $ATPH^-$	$6·89 \times 10^{-4}$ (±0·06)	$1·90 \times 10^{-3}$ (±0·05)	$4·20 \times 10^{-3}$ (±0·07)	
Cu: ATP^{2-}	$7·53 \times 10^{-4}$ (±0·08)	$2·15 \times 10^{-3}$ (±0·10)	$4·80 \times 10^{-3}$ (±0·09)	
TCu(OH): ATP*	3·94†	10·96†	23·99†	

*TCu(OH): ATP = [Cu(OH): ATP^{3-}] + [$Cu(OH)_2$: ATP^{4-}] + [$(Cu(OH): ATP)_2^{6-}$].
†Overall *p*H independent rate constants.

TABLE 3 — ACTIVATION PARAMETERS* FOR THE UNCATALYSED AND Cu(II) CATALYSED ATP HYDROLYSIS

[μ = 0·1 (KNO_3)]

ATP species	$\Delta H^{\ddagger}$ (kcal/mole)	$\Delta S^{\ddagger}$ (e.u.)	$\Delta F^{\ddagger}$ (kcal/mole)
H_2ATP^{3-}	31·2	10·9	27·6
$HATP^{3-}$	27·8	0·3	27·7
ATP^{4-}	20·9	−24·0	28·7
Cu: $ATPH^-$	21·6	−13·7	26·1
Cu: ATP^{2-}	22·0	−12·2	26·0
TCu(OH): ATP†	21·6	3·6	20·4

*Reference temperature = 56°.
†TCu(OH): ATP = [Cu(OH): ATP^{3-}] + [$Cu(OH)_2$: ATP^{4-}] + [$(Cu(OH): ATP)_2^{6-}$].

TABLE 4 — EXPERIMENTAL AND CALCULATED RATE CONSTANTS FOR THE Cu(II) CATALYSED ATP HYDROLYSIS

[μ = 0·1 (KNO_3)]

pH	46° (a)		56° (b)		64° (b)	
	Expl	Calc.	Expl	Calc.	Expl	Calc.
3·0	1·34	1·36	0·37	0·38	0·84	0·85
3·5	2·80	2·85	0·73	0·76	1·70	1·73
4·0	4·55	4·50	1·29	1·24	2·76	2·71
4·5	5·71	5·65	1·63	1·56	3·46	3·40
5·5	60·10	5·42	19·95	1·40	41·71	2·81
6·0	39·80	3·49	10·36	0·81	26·61	1·48
6·5	21·10	1·39	7·57	0·28	11·06	0·47
7·0	14·13	0·33	4·27	0·06	6·49	0·09
7·5	7·94	0·05	1·76	0·01	4·41	0·01
8·0	4·17	0·01	1·13	0·001	2·15	0·001

(a) Rate constant, $min^{-1} \times 10^4$ and (b) rate constant, $min^{-1} \times 10^3$.

TABLE 5 — MOLE FRACTIONS OF THE VARIOUS Cu:ATP CHELATE SPECIES IN SOLUTION

[Temp. = 46°; μ = 0·1 (KNO_3)]

pH	Cu: $ATPH^-$	Cu: ATP^{2-}	Cu: (OH) ATP^{3-}	Cu: $(OH)_2$ ATP^{4-}	(Cu: (OH) $ATP)^{6-}$
3·0	0·153	0·041			
3·5	0·214	0·446			
4·0	0·166	0·446			
4·5	0·080	0·677	0·018		
5·0	0·029	0·776	0·064		
5·5	0·008	0·712	0·187	0·009	0·014
6·0		0·464	0·386	0·056	0·058
6·5		0·185	0·486	0·222	0·092
7·0		0·044	0·367	0·531	0·052
7·5		0·007	0·176	0·803	0·012
8·0		0·001	0·064	0·932	0·002

*p*H region 3-9 gave a single value for each of the three ionic species of ATP. These values and the corresponding values of the activation parameters are listed in Tables 2 and 3 respectively.

First order rate constants for the kinetic runs carried out with equimolar amounts of Cu(II) and ATP are given in Table 4. These constants have been corrected for the spontaneous hydrolysis of the uncomplexed ATP at a particular *p*H. The rate of the Cu(II) catalysed ATP hydrolysis was found to increase with *p*H and an optimum value was found around *p*H 5·50. The rate then decreases with increasing *p*H. The data in Tables 1 and 2 show that in the presence of Cu(II) ions, the rate of ATP hydrolysis is higher than the uncatalysed rate, over the entire *p*H range of 3-8.

The thermodynamic data of Taqui Khan and Martell[18] have been used to calculate the concentrations of the various Cu:ATP chelate species in solution at 46°, 56° and 64°. From the concentrations of these various species, the corresponding mole fractions have been calculated. The values obtained at 46° are listed in Table 5. The overall experimentally evaluated first order rate constants in the *p*H region 3-5 (Table 4), have been resolved into the specific rate constants relating to the catalytically active monoprotonated (Cu:$ATPH^-$) and normal (Cu:ATP^{2-}) chelate species, and are listed in Table 2. Correlation between the experimental rate constants and the calculated rate constants in the *p*H region 3-5 (obtained from the specific rate constants of the monoprotonated and normal chelate species) is good, as seen from Table 4

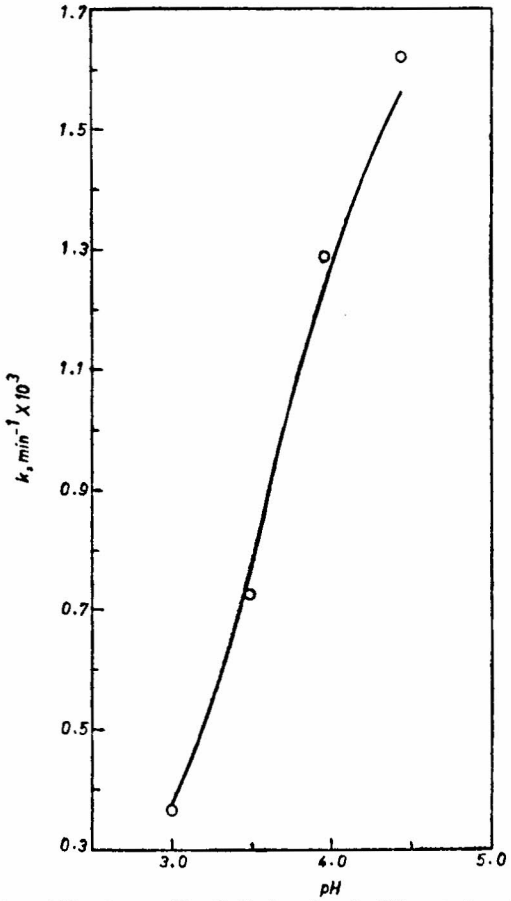

Fig. 1 — pH-rate profile (3-5) for the Cu(II) catalysed ATP hydrolysis [Temp. = 56·0°, μ = 0·1 (KNO_3). Solid line— calculated rates; ○ — measured rates. Similar pH-rate profile was obtained at 46° and 64°]

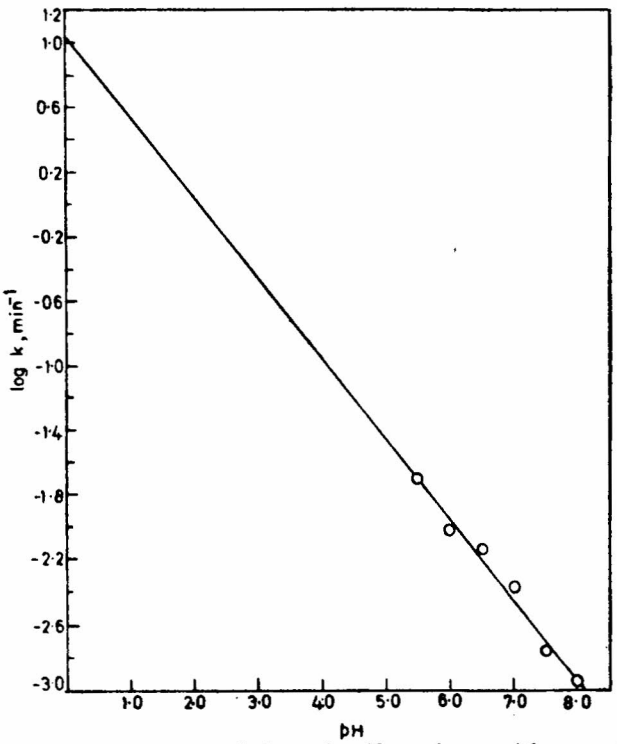

Fig. 2 — Evaluation of the order (dependence with respect to hydrogen ion concentration) in the Cu(II) catalysed ATP hydrolysis [Temp. = 56°, μ = 0·1 (KNO_3). Similar plots were obtained at 46° and 64°]

and Fig. 1. At pH >5 the hydrolysis reaction is found to have a 1/2 order dependence with respect to [H^+] as shown in Fig. 2. An overall pH independent hydrolysis rate constant pertaining to the Cu:ATP hydroxospecies has been evaluated and is listed in Table 2.

Activation parameters $\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$ and $\Delta F^{\ddagger}$ related to the specific rate constants of the catalytically active Cu:ATP species were determined by studying the kinetics of the Cu(II) catalysed ATP hydrolysis at 46°, 56° and 64°. These activation parameters are listed in Table 3.

Discussion

Uncatalysed ATP hydrolysis — The kinetics of the spontaneous hydrolysis of ATP is given by the overall rate law

$$\frac{-dL}{dt} = k_{obs}[T_L]$$

where

$$T_L = [H_2ATP^{2-}] + [HATP^{3-}] + [ATP^{4-}]$$

and

$$k_{obs} = k_1M_1 + k_2M_2 + k_3M_3$$

k_1, k_2 and k_3 are the specific rate constants and M_1, M_2 and M_3 are the corresponding mole fractions of the H_2ATP^{2-}, $HATP^{3-}$ and ATP^{4-} species, respectively.

The three ionic forms of ATP present in the solution in the pH range 3-9 are shown in Fig. 3. The specific rate constants for these three ionic forms of ATP, listed in Table 2, show that the diprotonated form, H_2ATP^{2-}, is the most kinetically active, followed by the monoprotonated species, $HATP^{3-}$. The fully dissociated form, ATP^{4-} is kinetically the slowest of the three species.

It is difficult to specify precisely, the mechanisms by which the various forms of ATP undergo hydrolysis, because for the conditions under which the reaction occurs, there is no appropriate criterion

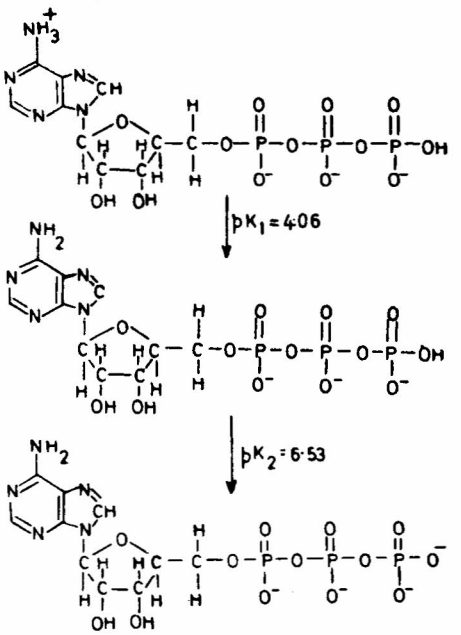

Fig. 3 — Dissociation steps for adenosine-5′-triphosphate

of molecularity with respect to water. The hydrolysis of a particular species of ATP may take place either by a S_N1 mechanism, involving the elimination of a metaphosphate intermediate, or by a S_N2 mechanism involving nucleophilic attack by a polar water molecule on the said ionic species of ATP in solution.

The entropies of activation, $\Delta S^{\ddagger}$, for the hydrolysis reactions of esters, are of much importance in distinguishing between the S_N1 and S_N2 mechanisms. From the study of the $\Delta S^{\ddagger}$ of a large number of ester hydrolysis reactions, Schaleger and Long[19] have postulated that S_N1 mechanisms are accompanied by small entropies of either sign, while the S_N2, proceed with large negative entropies of activation. The $\Delta S^{\ddagger}$ values for the hydrolysis of the three ionic species of ATP, listed in Table 3, show that the diprotonated and the monoprotonated species of ATP have positive values of 10·9 e.u. and 0·3 e.u. respectively, while the fully dissociated species has a $\Delta S^{\ddagger}$ value of $-24{\cdot}0$ e.u. In the light of the above observations, it may be postulated that the di- and mono-protonated species of ATP hydrolyse by S_N1 mechanism (Fig. 4A) while the fully dissociated form of ATP hydrolyses by a S_N2 mechanism (Fig. 4B). Further support for the above postulation is obtained from the work of Miller and Westheimer[20] on the hydrolysis of γ-phenylpropyl di-(ADP)- and tri-(ATP)-phosphates. The study was carried out at 95° in the *p*H range 0·3-10, various buffers being used to maintain a constant *p*H. These authors have shown that in the *p*H region 3-6, wherein the H_2ATP^{2-} and $HATP^{3-}$ species predominate, the hydrolysis reaction proceeds by the elimination of a monomeric metaphosphate anion. Further confirmation of the mechanism of hydrolysis can be obtained by a detailed investigation of the effect of ionic strength, effect of D_2O, effect of a nucleophile like pyridine, or by carrying out the reaction in a non-polar solvent like acetonitrile.

Cu(II) catalysed ATP hydrolysis — A detailed potentiometric study[21] has shown that the 1:1 metal-ATP complexes [where metal = Cu(II), Zn(II), Mn(II), Mg(II) and Ca(II)] have no affinity to further complex with another ATP molecule or a sulphosalicylic acid (SSA) molecule, to form a 1:2 metal-ATP or a 1:1:1 metal-ATP-SSA complexes, respectively. This lack of interaction is attributed to the electrostatic repulsion between the negatively charged 1:1 complexes and a second anionic ATP^{4-} or SSA molecule. Since 1:2 Cu-ATP complexes are not formed, the kinetics of the Cu(II)-catalysed ATP hydrolysis has been carried out with a 1:1 mole ratio of Cu(II) and ATP.

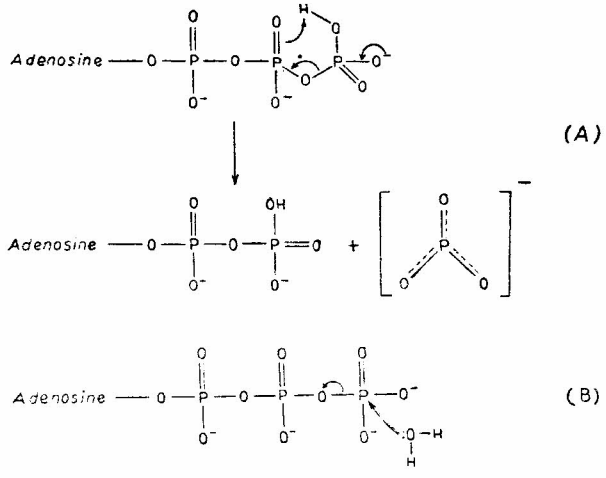

Fig. 4 — Mechanisms for the uncatalysed hydrolysis of adenosine-5′-triphosphate

Potentiometric study of 1:1 Cu-ATP systems[18] has shown that Cu(II) interacts strongly with ATP to give rise to a series of complexes, namely a protonated $Cu:ATPH^-$, a normal $Cu:ATP^{2-}$, a monohydroxo $Cu:(OH)ATP^{3-}$, a dihydroxo $Cu:(OH)_2ATP^{4-}$ and a dimeric species $[Cu:(OH)ATP]_2^{6-}$. The mole fractions of these various species at 46° (listed in Table 5) show that up to *p*H 5, the Cu:ATP species in solution predominently consist of $Cu:ATPH^-$ and $Cu:ATP^{2-}$ species only, the hydroxo species being $\lesssim 10\%$ of the total metal chelate species. Concentration of the hydroxo species increases rapidly with *p*H, and at *p*H 8 their concentration approaches 99% of the total Cu:ATP chelate species in solution. In the acidic *p*H range 3-5 the observed rate can be assigned to the predominant $Cu:ATPH^-$ and $Cu:ATP^{2-}$ species. In this *p*H range, the rate law for the hydrolysis of the Cu:ATP system is given by the relationship

$$\frac{-dL}{dt} = k_{obs}[T_L]$$

where

$$T_L = [Cu:ATPH^-] - [Cu:ATP^{2-}]$$

and

$$k_{obs} = k_1M_1 + k_2M_2$$

k_1 and k_2 are the specific rate constants and M_1 and M_2 are the corresponding mole fractions of the $Cu:ATPH^-$ and $Cu:ATP^{2-}$ species respectively. Comparison of the specific rate constants of the $Cu:ATPH^-$ and $Cu:ATP^{2-}$ species with those of the corresponding free ligand species $ATPH^{3-}$ and ATP^{4-} respectively (Table 2) shows that Cu(II) ions catalyse the rate of ATP hydrolysis considerably. The ratio of the rates for the catalysed and uncatalysed reaction of the above-mentioned species are about 12 and 60 respectively at 56°. Thus the metal ion catalysis is most effective for the fully dissociated form of ATP, i.e. ATP^{4-}.

The catalytic activity of the Cu(II) ions on the rate of ATP hydrolysis can be directly attributed to the changes brought about in the charge of the ATP molecule and the polarization of the P-O bonds. On complexation with the ATP molecule, Cu(II) ions reduce the negative charge on the molecule by two units. Due to this screening of the negative charge on the phosphorous oxygen atoms, the approach of a nucleophile to the site of attack, will be greatly facilitated leading to enhanced rates of hydrolysis. Further, complexation of the Cu(II) ions with the oxygen atoms of the phosphate chain will result in the withdrawal of electrons from the oxygen atoms towards the sites of metal binding. This leads to polarization of the P-O bonds with an enhancement of positive charge density on the phosphorous atom, and a facile nucleophilic attack on the polarized phosphorous atom.

The data in Table 2 show that the normal complex species Cu:ATP^{2-} reacts at a slightly faster rate than the monoprotonated species Cu:ATPH^{-}. The latter species has the advantage of a lower, negative charge and the gamma phosphorous atom at the site of attack is polarized by both the metal ion and the proton. However, the much greater interaction of Cu(II) ions in the Cu:ATP^{2-} species (log $K_{ML} = 5{\cdot}83$ at 56°) leading to a greater polarization of the P-O bond, is more than enough to compensate for the lower negative charge and the combined polarizing effect in the Cu:ATPH species (log $K_{MHL} = 2{\cdot}91$ at 56°).

The entropies of activation, for the hydrolysis of the monoprotonated and the normal chelate species are −13·7 e.u. and −12·2 e.u. respectively (Table 3). Entropies of this order of magnitude are associated with hydrolysis reactions that proceed by a S_N2 mechanism[19]. The monoprotonated uncomplexed ATPH^{3-} species has been suggested to undergo hydrolysis by a S_N1 mechanism, involving the transfer of a proton to the leaving group, and elimination of the metaphosphate anion (Fig. 4A). In the corresponding monoprotonated metal complex, Cu:ATPH^{-}, the negative charge on the gamma phosphorous atom, which is the driving force behind the S_N1 mechanism for the ATPH^{3-} species, is absent. Further complexation of the metal ion with the oxygens of the beta and gamma phosphates[22,23] hinders the free rotation of the, terminal phosphate moiety, and the transfer of a proton to the leaving group, required for the S_N1 mechanism, is thus rendered difficult. Due to these reasons the Cu:ATPH^{-} species seems to undergo hydrolysis via a S_N2 mechanism.

For the normal complex species, Cu:ATP^{2-}, the entropy of activation for hydrolysis (−12·2 e.u.) is much more positive than the value (−24 e.u.) observed for the fully dissociated species, ATP^{4-}. If the Cu:ATP^{2-} species is also assumed to react with the nucleophile : OH_2 by a S_N2 mechanism, the more positive entropy of activation for the metal chelate species as compared to the corresponding uncomplexed ATP^{4-} species, may be readily explained on the basis of the decreased repulsion between the Cu:ATP^{2-} species and the attacking polar water molecule. In the light of the above discussion, the hydrolysis of ATP in the presence of Cu(II) ions can be visualized as a S_N2 nucleophilic attack of a polar water molecule on the terminal phosphate moiety of ATP, as evidenced by the work of Moll *et al.*[24]. These proposed mechanisms are diagramatically shown in Figs. 5A and B.

Above *p*H 5, the hydrolysis reaction mechanism is complicated by the formation of hydroxo species. After correction for the contribution of the Cu:ATPH^{-} and Cu:ATP^{2-} species, the overall rate constants should be the sum of the contributions of the monohydroxo, dihydroxo and dimeric species of ATP. Equations assuming a simple first order dependence on the substrate species did not give any constant values for the specific rate constants of the three hydroxo species. Unlike the acidic region, the mechanism of hydrolysis must involve H^+ or OH^- ions in the *p*H region >5·5. The plot of log *k* against *p*H (Fig. 2), is linear with a slope of 1/2,

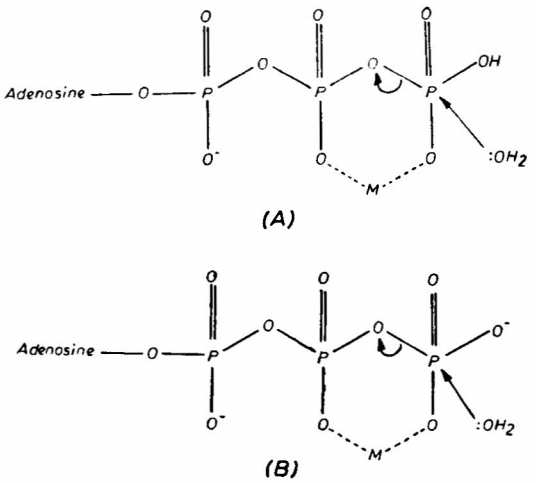

Fig. 5 — Mechanisms of hydrolysis for the protonated and normal Cu: ATP chelate species

showing a 1/2 order dependence on the [H^+]. The intercept of the above plot gives an overall (*p*H independent) hydrolysis rate constant. The rate law for the hydrolysis of ATP, via the hydroxo species in the *p*H range 5·5-·80 may then be represented by

$$\frac{-dL}{dt} = k[T_L][H^+]_{1/2}$$

where

$$T_L = [\text{Cu:(OH)ATP}^{3-}] + [\text{Cu:(OH)}_2\text{ATP}^{4-}] + [(\text{Cu:(OH)ATP}_2^{6-}]$$

and

k = overall *p*H independent hydrolysis constant.

The rate constants obtained at 46°, 56° and 64°, and the corresponding activation parameters are listed in Tables 2 and 3. In view of the complex dependence on H^+ further resolution of the overall rate constants into individual rate constants for the various hydroxo species was not possible with the data on hand. The overall entropy and enthalpy of activation for these species (Table 3) do not lead to any precise mechanism for any one individual hydroxo species.

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